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生长温度对烟草叶片环式电子传递活性的影响*

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摘要: 高等植物的光合机构在环境胁迫条件下非常容易产生光抑制, 环式电子传递在光合机构的光保护中发挥着重要的作用。但是, 生长温度对环式电子传递的影响并不清楚。本研究测定了在 24/18 ℃和 32/26 ℃条件下生长 40 天的烟草 (K326) 叶片的气体交换、叶绿素荧光和 P700 氧化还原态的光响应曲线。结果表明, 烟草叶片在两种生长温度下的的光合能力、光化学淬灭、非光化学淬灭和通过光系统 II 的电子传递速率 (ETR II) 均没有差异。但是, 在强光条件下, 生长在 24/18 ℃的叶片比生长在 32/26 ℃的具有更高的通过光系统 I 的电子传递速率 (ETR I) 和 ETR I/ETR II 比值。短时间的强光处理后, 生长在 24/18 ℃的叶片具有较高的光系统 II 最大量子产额 (F_v/F_m), 表明环式电子传递活性的上调有助于缓解生长在 24/18 ℃的叶片光系统 II 受到的光损伤。综上所述, 环式电子传递活性的增强是植物适应较低生长温度的重要策略。

关键词: 环式电子传递; 生长温度; 光抑制; 光保护; 光系统 II
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Effect of Growth Temperature on the Activity of Cyclic Electron Flow in Tobacco Leaves

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Abstract: Cyclic electron flow (CEF) around photosystem I (PSI) is an important mechanism for photoprotection in higher plants under environmental stresses. However, the response of CEF activity to growth temperature has not been clarified. We here monitored gas exchange, chlorophyll fluorescence, and the P700 redox state over a range of light intensities in leaves of tobacco cultivar 'k326' grown at 24/18 ℃ and 32/26 ℃ (day/night). No significant difference was found in the capacity of photosynthetic CO_2 assimilation between the plants grown at 24 ℃ and 32 ℃. In addition, the light response changes in the photochemical quenching of photosystem II (Y(II)) and non-photochemical quenching (NPQ) did not differ significantly between those plants. Light response curves indicated that the plants grown at 24 ℃ and 32 ℃ displayed the same level of electron flow through PSII (ETR II) irrespective of light intensity. However, under intense light, plants grown at 24 ℃ showed significantly higher electron flow through PSI (ETR I). The ETR I/ETR II ratio was significantly higher in plants grown at 24 ℃ when exposed to intense light. Furthermore, after short-term treatment with strong light at 24 ℃, the maximum quantum yield of photosystem II (F_v/F_m) was significantly higher in plants grown at 24 ℃ than that grown at 32 ℃. Taken together, our results suggest that enhancement of CEF activity in plants grown at 24 ℃ alleviates PSII photoinhibition, which is an important strategy in tobacco for acclimating to a relatively low growth temperature.

Key words: Cyclic electron flow; Growth temperature; Photoinhibition; Photoprotection; Photosystem II

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Plants capture light energy through light-harvesting systems. The absorbed energy then drives photosynthetic electron flow through the thylakoid membranes of the chloroplasts. Electrons pass through the cytochrome b_6/f complex generate a proton gradient (ΔpH) across the thylakoid membrane. The formation of ΔpH drives ATP synthesis by the ATP synthase complex. Products of the light reactions, ATP and NADPH, are ultimately used in the Calvin-Benson and photorespiratory cycles. Environmental stresses can decrease stomatal conductance, and then limit the operation of photosynthetic CO_2 fixation (Murata *et al.*, 2007). A constraint in the capacity of the Calvin-Benson cycle can increase the ratio of NADPH/$NADP^+$ and depress the operation of photosynthetic electron flow from photosystem I (PSI) to $NADP^+$. That then leads to the production of reactive oxygen species (ROS) that can accelerate photoinhibition of photosystem II (PSII) (Murata *et al.*, 2007; Oguchi *et al.*, 2009, 2011). An increase in NADPH/$NADP^+$ ratio can activate cyclic electron flow (CEF) around PSI (Johnson, 2005; Shikanai *et al.*, 2007; Okegawa *et al.*, 2008), thereby enhancing the formation of ΔpH across thylakoid.

In higher plants, CEF is suggested to be essential for balancing the ATP/NADPH production and protecting PSI and PSII from photodamage under conditions in which the absorbed light is in excess of the photon requirements for photosynthesis (Takahashi *et al.*, 2009; Miyake, 2010; Takahashi and Badger, 2011). The CEF-dependent generation of ΔpH across the thylakoid membrane is necessary for activating non-photochemical quenching (NPQ) and preventing stromal over-reduction (Munekage *et al.*, 2002, 2004; Nandha *et al.*, 2007). Activation of NPQ can harmlessly dissipate excess light energy as heat and then diminish the production of ROS (Niyogi *et al.*, 1998, 2001). The main action of ROS in accelerating PSII photoinhibition can inhibit the repair of photodamaged PSII complex at the step of D1 protein synthesis (Nishiyama *et al.*, 2001, 2004, 2011). *Arabidopsis* mutants lacking PGR5-dependent CEF activity (*pgr5* mutants) show the same capacity of NPQ as those in which NPQ activity is absent (*npq1* and *npq4* mutants) (Takahashi *et al.*, 2009). However, *pgr5* mutants incur significantly more PSII photodamage than do *npq1* and *npq4* mutants. This indicates that CEF alleviates PSII photoinhibition through at least two independent mechanisms: one linked to NPQ that favors the repair of photodamaged PSII; the other one, independent of NPQ, suppresses photodamage to PSII (Takahashi *et al.*, 2009).

The effect of various environmental stresses on CEF activity has been studied extensively (Horvath *et al.*, 2000; Golding and Johnson, 2003; Li *et al.*, 2004; Wang *et al.*, 2006; Yamori *et al.*, 2011; Huang *et al.*, 2011, 2012). For example, CEF plays an important role in photoprotection under severe short-term stresses, including those associated with intense light, drought, and extreme temperatures (Li *et al.*, 2004; Wang *et al.*, 2006; Takahashi *et al.*, 2009; Huang *et al.*, 2011, 2012, 2013). Such conditions can induce inhibition of CO_2 assimilation and then lead to over-reduction of the electron transport chain. Several studies have reported that CEF activity could be enhanced by temporary heat stress (Havaux, 1996; Bukhov *et al.*, 1999; Kou *et al.*, 2013) or be accelerated by brief exposure to chilling temperatures, as noted with tobacco, cucumber, and tropical tree species (Kim *et al.*, 2001; Barth and Krause, 2002; Huang *et al.*, 2011). However, the response of CEF capacity to long-term changes in temperature is unclear.

Temperature is a major limiting factor affecting the distribution of plants. Photosynthesis has long been recognized as one of the most temperature-sensitive processes in plants. Because low temperature can induce stomatal closure and lead to PSII photoinhibition, plants grown under such conditions probably have high CEF capacity to increase the generation of a proton gradient across the thylakoid membrane (ΔpH) and alleviate this photoinhibition. Here, we examined the effect of growth temperature

on CEF capacity. Plants of *Nicotiana tabacum* (tobacco) cultivar 'k326' were cultured at 24/18 ℃ and 32/26 ℃ (day/night). We hypothesize that tobacco plants regulate CEF activity to acclimate to growth temperature and then protect PSII against photoinhibition.

1 Materials and methods

1.1 Plants materials and growth conditions

The seedlings of tobacco cultivar 'k326' were cultivated in plastic pots, then transferred to two phytotrons set to either 32/26 ℃ or 24/18 ℃ (day/night), hereafter referred to as 32 ℃ or 24 ℃, respectively. These phytotrons, located at Kunming, Yunnan, China (elevation 1 900 m; 102°41′E, 25°01′N) relied upon sunshine as the light source, with plants receiving approximately 95% of full sunlight. During the experimental period, none of the plants experienced any water or nutrient stresses. The relative humidity was kept at 60% and the atmospheric CO_2 concentration was maintained at 400 μmol mol^{-1}. During 40 and 60 days after transplantation, mature leaves were used for photosynthetic measurements.

1.2 Measurement of chlorophyll content

Contents of chlorophyll a and b were determined according to the method of Inskeep and Bloom (1985).

1.3 Gas exchange measurement

Rates of CO_2 assimilation (A_n) were measured at 24 ℃ with an open gas exchange system that incorporated infrared CO_2 and water vapor analyzers (Li-6400; Li-Cor Inc, Lincoln, NE, USA). During the measurements, the relative air humidity was 60% and atmospheric CO_2 concentration was 400 μmol mol^{-1}. The leaf samples were illuminated by either a quartz halogen light source or red light-emitting diodes (656 - 680 nm; Li-6400-02, Li-Cor Inc). Measurements of A_n in response to incident photosynthetic photon flux density (PPFD) were made between 2 000 and 0 μmol photons m^{-2} s^{-1} with a relative air humidity of 60%. Curves for the rate of CO_2 assimilation to intercellular concentration of CO_2 (A/C_i) were measured between 2 000 and 0 mmol mol^{-1} atmospheric CO_2 concentration while PPFD was maintained at 1 000 μmol photons m^{-2} s^{-1} (von Caemmerer and Farquhar, 1981). Based on those A/C_i curves, we calculated the maximum rates of RuBP regeneration (J_{max}) and RuBP carboxylation (V_{cmax}) according to the method of Long and Bernacchi (2003).

1.4 Determinations of chlorophyll fluorescence and the P700 redox state

Using a Dual-PAM-100 Measuring System (Heinz Walz, Effeltrich, Germany) connected to a computer with control software, we conducted synchronous measurements for the light responses of chlorophyll fluorescence and the P700 redox state in the 24 ℃ phytotron. The relative air humidity was 60% and atmospheric CO_2 concentration was 400 μmol mol^{-1}. Five mature leaves were light-adapted (1 000 μmol photons m^{-2} s^{-1}) for at least 20 min to induce stomata opening prior to determining the light response curves. Values for light-adapted photosynthetic parameters were recorded after 3 min of exposure to 1 976, 1 618, 1 311, 1 052, 849, 555, 363, 240, and 119 μmol photons m^{-2} s^{-1}. The fluorescence parameters were calculated as follows:

$$F_v/F_m = (F_m - F_o)/F_m$$

$$F_o' = F_o/(F_v/F_m + F_o/F_m')$$

(Oxborough and Baker, 1997)

$$qL = (F_m' - F_s)/(F_m' - F_o') \times F_o'/F_s$$

(Baker, 2008)

$$Y(II) = (F_m' - F_s)/F_m' \text{ (Genty, 1989)}$$

$$Y(NPQ) = F_s/F_m' - F_s/F_m \text{ (Kramer } et\ al.\text{, 2004)}$$

$$Y(NO) = F_s/F_m \text{ (Hendrickson } et\ al.\text{, 2004; Kramer } et\ al.\text{, 2004)}$$

where F_v/F_m represents the maximum quantum yield of PSII after dark adaptation, making it a useful indicator for estimating PSII activity; qL represents the proportion of PSII centers in the open state (with oxidized primary quinone acceptor Q_A); Y(II) is the effective quantum yield of PSII; Y(NPQ) is the fraction of energy dissipated as heat via the regulated non-photochemical quenching mechanism; and Y(NO)

is the fraction of energy that is passively dissipated in the forms of heat and fluorescence.

The P700 redox state was measured with a dual wavelength unit (830/875 nm) according to the method of Klüghammer and Schreiber (2008). Saturation pulses (10 000 μmol photons m^{-2} s^{-1}) were also applied to assess the P700 parameters (Klüghammer and Schreiber, 1994, 2008). The $P700^+$ signals (P) can vary between a minimal (P700 fully reduced) and a maximal level (P700 fully oxidized). The maximum level, P_m, was determined with application of a saturation pulse after pre-illumination with far-red light. P_m' was determined similarly to P_m, but with background actinic light instead of far-red illumination. The photochemical quantum yield of PSI, i.e., Y(I), was calculated as $Y(I)=(P_m'-P)/P_m$. Because ETRII is responsible for linear electron flow (LEF) and ETRI involves LEF and CEF, if CEF were activated, the value of ETRI would be higher than the value of ETRII. As a result, the higher value of ETRI than that of ETRII is regarded as an indicator of CEF activation (Huang *et al.*, 2011, 2012, 2013; Yamori *et al.*, 2011). Here, the light response change in ETR I/ETR II ratio was used to compare the CEF activity in plants grown at 24 ℃ versus 32 ℃.

1.5 Photoinhibitory treatment

Detached leaves were placed on moist papers and treated at 2 000 μmol photons m^{-2} s^{-1}, 24 ℃, and an atmospheric CO_2 concentration of 400 μmol mol^{-1}. Before and after exposure to highlight, the value of F_v/F_m was measured via Dual-PAM-100 Measuring System following 20 min of dark-incubation at 24 ℃.

1.6 Statistical analysis

The results were displayed as mean values of at least five individuals. A one-way ANOVA was performed ($\alpha=0.05$) to determine any significant differences among treatments.

2 Results

The light response curves indicated that, under intense illumination, stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were higher in plants grown at 24 ℃ compared with those grown at 32 ℃ (Fig. 1A, B). Photosynthetic CO_2 assimilation (A_n) did not significantly differ between the plants grown at 24 ℃ and 32 ℃ (Fig. 1C). Moreover, at 1 000 μmol photons m^{-2} s^{-1}, the response of CO_2 assimilation to C_i did not differ significantly in leaves grown at 24 ℃ and 32 ℃ (Fig. 2). The value of J_{max} was 84 μmol m^{-2} s^{-1} and 78 μmol m^{-2} s^{-1} in leaves grown at 24 ℃ and 32 ℃, respectively (Fig. 3A). Values for J_{max} were 84 μmol m^{-2} s^{-1} and 78 μmol m^{-2} s^{-1} in leaves grown at 24 ℃ and 32 ℃, respectively, while those for V_{cmax} were 78 μmol m^{-2} s^{-1} and 76 μmol m^{-2} s^{-1}, respectively (Fig. 3A). Their corresponding J_{max}/V_{cmax} ratios were 1.08 and 1.03. Furthermore, J_{max}, V_{cmax}, and the J_{max}/V_{cmax} ratio were not significantly changed in plants grown at 24 ℃ and 32 ℃. Although the content of chlorophyll *b* in plants grown at 24 ℃ was slightly higher than that at 32 ℃, the contents of chlorophyll *a* and total chlorophyll were not significantly altered in response to temperature (Fig. 3B). Therefore, these results indicated that the plants grown at both temperatures had the similar capacity to capture light energy and assimilate CO_2.

Light response changes in Y(II), Y(NPQ), and Y(NO) did not vary significantly between plants grown at 24 ℃ and 32 ℃ (Fig. 4A–C), indicating that plants grown at both temperatures were equally capable of utilizing and dissipating absorbed light energy in PSII. Furthermore, plants grown at 24 ℃ and 32 ℃ showed the same value of 1-qL under all light intensities (Fig. 4D), indicating no alterations occurred in the stromal redox state between them.

Light response curves indicated that ETRII did not differ between plants grown at 24 ℃ and 32 ℃, irrespective of light intensity (Fig. 5A). Plants grown at 24 ℃ and 32 ℃ showed the same value of ETRI under light intensities below 1 052 μmol photons m^{-2} s^{-1} (Fig. 5B). However, when illuminated at light intensities above 1 311 μmol photons m^{-2} s^{-1},

the value of ETRI was significantly higher in plants grown at 24 ℃ than that grown at 32 ℃ (Fig. 5B). Under 1 618 μmol photons m^{-2} s^{-1}, ETR I was 245 and 216 μmol electrons m^{-2} s^{-1} in plants grown at 24 ℃ and 32 ℃, respectively. The value of ETR I under 1 976 μmol photons m^{-2} s^{-1} was 251 and 216 μmol electrons m^{-2} s^{-1} in plants grown at 24 ℃ and 32 ℃, respectively. Light response change in ETR I/ETR II ratio is regarded as an indicator of activation of CEF. Plants grown at 24 ℃ and 32 ℃ showed no significant difference of ETR I/ETR II ratio when exposed to light intensities below 1 311 μmol photons m^{-2} s^{-1} (Fig. 5C). When exposed to light intensities of 1 618 and 1 976 μmol photons m^{-2} s^{-1}, the ETR I/ETR II ratio was significantly higher in plants grown at 24 ℃ than that grown 32 ℃ (Fig. 5C). These results indicated that plants grown at 24 ℃ had significantly greater CEF activity than that grown at 32 ℃.

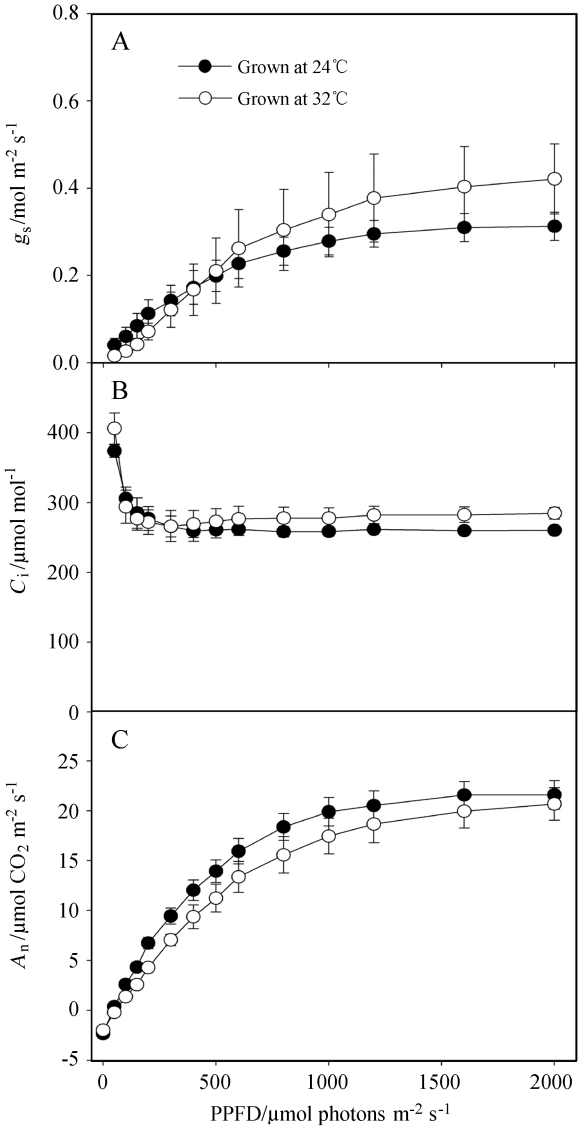

Fig. 1　Responses of stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and CO_2 assimilation (A_n) to incident photosynthetic photon flux density (PPFD) in leaves of tobacco grown at 24 ℃ and 32 ℃. Values are means ± *SE* (n = 5)

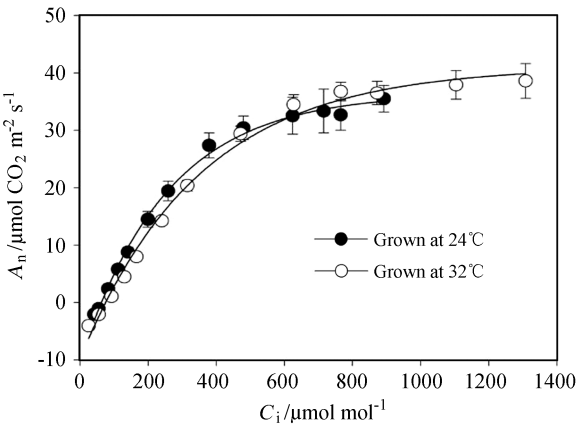

Fig. 2　Response of CO_2 assimilation (A_n) to incident intercellular CO_2 concentration (C_i) in leaves of tobacco grown at 24 ℃ and 32 ℃. Values are means ± *SE* (n = 5)

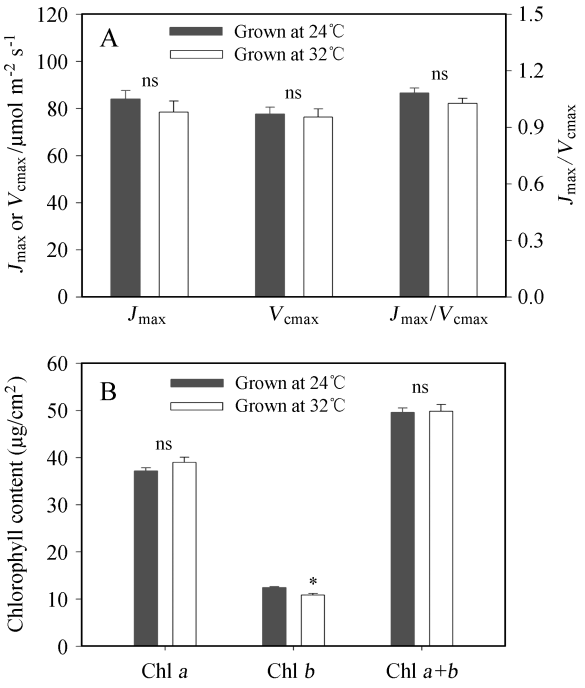

Fig. 3　Maximum rates of RuBP regeneration (J_{max}) and RuBP carboxylation (V_{cmax}), J_{max}/V_{cmax} ratio, and chlorophyll content in leaves of tobacco grown at 24 ℃ and 32 ℃. Values are means ± *SE* (n = 4 ~ 5). Significant differences (shown by asterisks) between plants grown at 24 ℃ and 32 ℃ were examined via one-way ANOVA (P < 0.05)

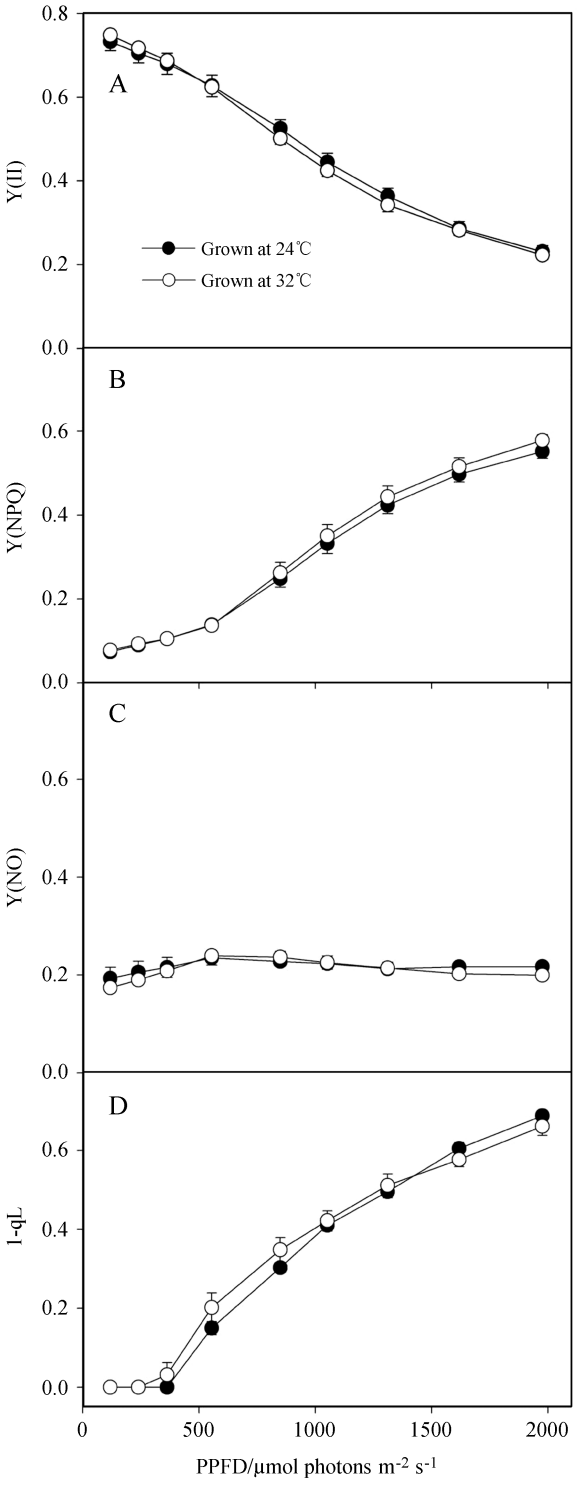

Fig. 4 Responses of Y(II), Y(NPQ), Y(NO), and 1-qL to incident photosynthetic photon flux density (PPFD) in leaves of tobacco grown at 24 ℃ and 32 ℃. Y(II), effective quantum yield of PSII; Y(NPQ), fraction of energy dissipated as heat via regulated non-photochemical quenching mechanism; Y(NO), fraction of energy passively dissipated in forms of heat and fluorescence; qL, proportion of PSII centers in open state. Values are means ± *SE* (n = 4~5)

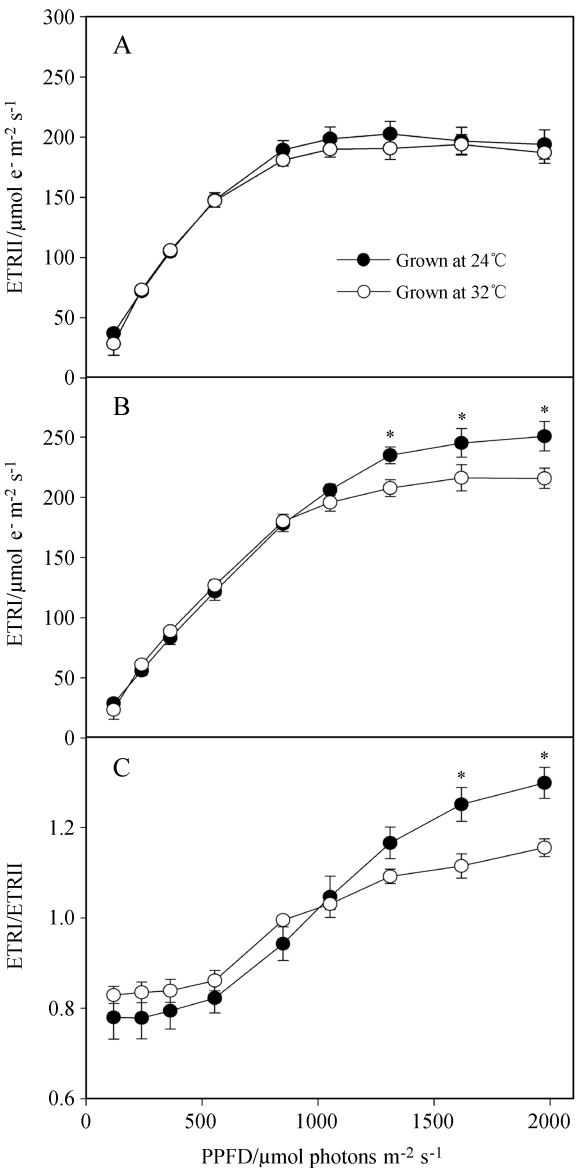

Fig. 5 Responses of ETRII, ETRI, and ETRI/ETRII ratio to incident photosynthetic photon flux density (PPFD) in leaves of tobacco grown at 24 ℃ and 32 ℃. Values are means ± *SE* (n = 4~5). Significant differences (shown by asterisks) between plants grown at 24 ℃ and 32 ℃ were examined via one-way ANOVA (P < 0.05)

When plants normally grown at 24 ℃ were treated for 1 h at 24 ℃ and 2 000 μmol photons m^{-2} s^{-1}, their leaf values of F_v/F_m decreased from 0.78 to 0.57 versus a decline from 0.78 to 0.50 for plants normally grown at 32 ℃ (Fig. 6). After 2 h of treatment with this highlight intensity, those F_v/F_m values decreased to 0.47 and 0.39 in plants normally grown at 24 ℃ and 32 ℃, respectively (Fig. 6). This

demonstrated that, under strong illumination at 24 ℃, plants originally exposed to 32 ℃ had significantly more PSII photoinhibition than those that had remained at 24 ℃ throughout this experimental period.

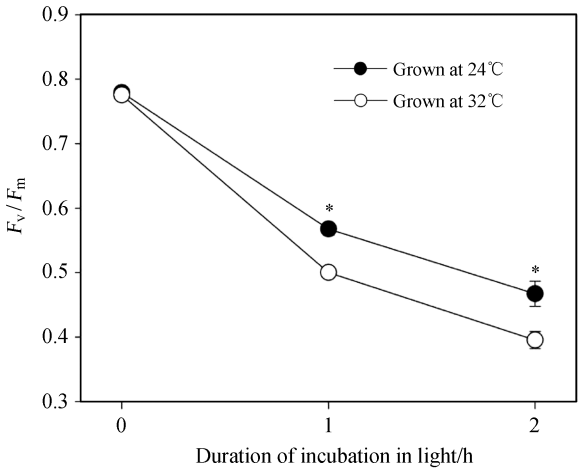

Fig. 6　Effect of high light on PSII photoinhibition in leaves of tobacco grown at 24 ℃ and 32 ℃. Detached leaves were exposed to light (2 000 μmol photons m^{-2} s^{-1}) at 24 ℃. Values are means ± *SE* (n=5). Asterisks indicate significant differences between plants grown at 24 ℃ and 32 ℃ (one-way ANOVA, P < 0.05)

3　Discussion

We hypothesized that the plants of tobacco could regulate CEF activity for acclimating to growth temperature. Light response curves showed that, under high light, the plants grown at 24 ℃ significantly had higher values of ETRI and ETR I/ETRII ratio than plants grown at 32 ℃. These results suggested that CEF activity was up-regulated in plants grown at 24 ℃ compared with that grown at 32 ℃. Because 24 ℃ is lower than the optimum growth temperature for tobacco (about 30 ℃, Yamori *et al.*, 2010), our results strongly suggest that the enhancement of CEF activity is an important strategy for tobacco plants to acclimate to relatively low growth temperature.

The response of CO_2 assimilation to incident light and C_i did not differ significantly between plants grown at 24 ℃ and 32 ℃ (Fig. 1 and Fig. 2). Likewise, levels of chlorophyll and values for J_{max}, V_{cmax}, and the J_{max}/V_{cmax} ratio were not significantly altered (Fig. 3). Based on these results we could conclude that 1) N-partitioning between the enzymes related to RuBP carboxylation and regeneration did not change in plants grown at either 24 ℃ or 32 ℃, and 2) the Rubisco activation state did not differ significantly between plants grown at these two temperatures. Furthermore, the light response change in 1-qL did not significantly differ for plants at 24 ℃ or 32 ℃ (Fig. 4D). Thus, the higher CEF activity in plants grown at 24 ℃ did not affect photosynthetic capacity and the stromal redox state. The enhancement of CEF activity in plants grown at 24 ℃ probably had other physiological functions, such as photoprotection for PSII.

The photoinhibition of PSII is a net result when the rate of photodamage exceeds that of repair. This repair of photodamaged PSII is based on the newly synthesis of D1 protein, which can be inhibited by ROS (Nishiyama *et al.*, 2001, 2004, 2011; Takahashi *et al.*, 2007, 2009). Because we found that the value of Y(NPQ) under high light did not differ in plants grown at 24 ℃ and 32 ℃, we assumed that ROS production was equal. Consequently, the rate of PSII recovery probably did not differ between the two temperature scenarios. Because ROS not only inhibits the repair of the photodamaged PSII complex but also accelerates the rate at which PSII is damaged (Oguchi *et al.*, 2009, 2011), we would have expected to find no growth-temperature-related change in the rate of ROS-induced PSII photodamage. After high-light treatment and dark-adaptation for 20 min at 24 ℃, F_v/F_m values were higher for plants grown at 24 ℃ than for those grown at 32 ℃ (Fig. 6). Therefore, plants at the lower temperature showed less rate of PSII photodamage. Photodamage to PSII occurs primarily at the oxygen-evolving complex (OEC), which is on the lumenal side of the thylakoid membrane (Hakala *et al.*, 2005; Ohnishi *et al.*, 2005). Following photodamage to the OEC, the supply of electrons from water to the primary electron donor of PSII ($P680^+$) is blocked, such that the level of $P680^+$ remains high (Takahashi and Murata, 2008; Takahashi and Badger, 2011). $P680^+$ is a strong oxidant that, at high levels, can damage the

PSII reaction centers (Anderson and Chow, 2002). Therefore, the difference in F_v/F_m after high-light treatment found between plants grown at 24 ℃ and 32 ℃ (Fig. 6) may have been caused by variability in the sensitivity of OEC to photodamage.

Recovery from inactivation of the OEC can be suppressed by calcium-channel blockers, thereby indicating that stability of that complex is dependent upon the Ca^{2+} in the lumen of the thylakoid membrane (Kerieger and Weis, 1993). Acidification of the lumen could drive a Ca^{2+}/H^+ antiport to sequester Ca^{2+} in the lumen. This has been demonstrated by Ettinger *et al.* (1999), who monitored the movement of approximately 4 mM Ca^{2+} into the lumen from an external concentration of 15 μM. Therefore, one might speculate that the generation of ΔpH is necessary for the stabilization of OEC. Furthermore, the formation of ΔpH through PGR5-dependent CEF activity can suppress PSII photodamage in *Arabidopsis* regardless of NPQ activation (Takahashi *et al.*, 2009). Once this OEC photodamage is alleviated by the CEF-dependent generation of ΔpH, the PSII reaction centers can be further protected. Therefore, the enhancement of CEF activity in plants grown at 24 promoted the generation of ΔpH under intense illumination and suppressed photodamage to PSII activity.

Field-grown plants are frequently exposed to low temperatures and high light intensities. Low temperatures decrease the stomatal conductance and activity in the Calvin-Benson cycle, thereby inducing the over-accumulation of NADPH and an over-reduction of photosynthetic electron chains under strong light (Murata *et al.*, 2007). Under such conditions, an increase in NADPH/$NADP^+$ ratio causes activation of NDH-dependent and PGR5-dependent CEF pathways (Johnson, 2005; Shikanai, 2007). The higher CEF activity in plants grown at 24 ℃ may be part of a strategy used by cold-sensitive species such as tobacco to acclimate to relatively low growth temperatures. Clear biochemical and spectroscopic evidence has been found in higher plants for the occurrence of at least two CEF pathways: PGR5 and NDH (Shikanai *et al.*, 1998; Munekage *et al.*, 2002, 2004; Johnson, 2011). Although our results indicated that the plants grown at the lower temperature had greater CEF activity, it is unclear which pathway was up-regulated. In rice, NDH-dependent CEF activity has an important role in regulating photosynthesis at low temperatures (Yamori *et al.*, 2011). Several cold-tolerant species show substantial up-regulation of the NDH-complex in response to low growth temperature and high light (Teicher *et al.*, 2000; Streb *et al.*, 2005; Laureau *et al.*, 2013). However, the rate of NDH-dependent CEF is estimated to be very low and its contribution to the formation of a proton gradient is not significant (Munekage *et al.*, 2002, 2004; Okegawa *et al.*, 2008). The promotion of CEF activity under high light is similar between *Arabidopsis* mutants lacking NDH-dependent CEF (*ccr6* mutants) and the control type (Yamori *et al.*, 2011). The main role of NDH may be to regulate the rate of PGR5-dependent CEF by poising the redox state of intersystem electron carriers (Peltier and Cournac, 2002). Therefore, we speculate that the tobacco plants grown at 24 ℃ probably up-regulate the components involved in PGR5-dependent CEF to enhance the generation of ΔpH under more intense light.

In summary, our results suggested that PGR5-dependent CEF activity was up-regulated in tobacco plants grown at 24 ℃ compared with that grown at 32 ℃, which alleviated PSII photoinhibition under intense light. In natural environments, temperatures often fluctuate in seasons or days. When strong illumination is coupled with low temperature, PSII activity usually decreases. Enhancement of CEF activity at a relatively low growth temperature helps in the generation of ΔpH and protects PSII against photodamage under high light. This is regarded as an important strategy by chilling-sensitive plants to acclimate to relatively lower growth temperatures.

References:

Anderson JM, Chow WS, 2002. Structural and functional dynamics of plant photosystem II [J]. *Philosophical Transactions of the Royal*

Society B- Biological Sciences, **357**: 1421—1430

Baker NR, 2008. Chlorophyll fluorescence: a probe of photosynthesis *in vivo* [J]. *Annual Review of Plant Biology*, **59**: 89—113

Barth C, Krause HG, 2002. Study of tobacco transformants to assess the role of chloroplastic NAD (P) H dehydrogenase in photoprotection of photosystems I and II [J]. *Planta*, **216**: 271—279

Bukhov NG, Wiese C, Neimanis S *et al.*, 1999. Heat sensitivity of chloroplasts and leaves: leakage of protons from thylakoids and reversible activation of cyclic electron transport [J]. *Photosynthesis Research*, **59**: 81—93

Ettinger WF, Clear AM, Fanning KJ *et al.*, 1999. Identification of a Ca^{2+}/H^{+} antiport in the plant chloroplast thylakoid membrane [J]. *Plant Physiology*, **119**: 1379—1385

Genty B, Briantais JM, Baker NR, 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence [J]. *Biochimica et Biophysica Acta*, **990**: 87—92

Golding AJ, Johnson GN, 2003. Down-regulation of linear and activation of cyclic electron transport during drought [J]. *Planta*, **218**: 107—114

Hakala M, Tuominen I, Keranen M *et al.*, 2005. Evidence for the role of the oxygen-evolving manganese complex in photoinhibition of photosystem II [J]. *Biochimica et Biophysica Acta*, **1706**: 68—80

Havaux M, 1996. Short-term responses of photosystem I to heat stress [J]. *Photosynthesis Research*, **47**: 85—97

Hendrickson L, Furbank RT, Chow WS, 2004. A simple alternative approach to assessing the fate of absorbed light energy using chlorophyll fluorescence [J]. *Photosynthesis Research*, **82**: 73—81

Horvath EM, Peter SO, Joet T *et al.*, 2000. Targeted inactivation of the plastid *ndhB* gene in tobacco results in an enhanced sensitivity of photosynthesis to moderate stomatal closure [J]. *Plant Physiology*, **123**: 1337—1349

Huang W, Zhang SB, Cao KF, 2011. Cyclic electron flow plays an important role in photoprotection of tropical trees illuminated at temporal chilling temperature [J]. *Plant and Cell Physiology*, **52**: 297—305

Huang W, Yang SJ, Zhang SB *et al.*, 2012. Cyclic electron flow plays an important role in photoprotection for the resurrection plant *Paraboea rufescens* under drought stress [J]. *Planta*, **235**: 819—828

Huang W, Fu PL, Jiang YJ *et al.*, 2013. Differences in the responses of photosystem I and photosystem II of three tree species *Cleistanthus sumatranus*, *Celtis philippensis* and *Pistacia weinmannifolia* submitted to a prolonged drought in a tropical limestone forest [J]. *Tree Physiology*, **33**: 211—220

Inskeep WP, Bloom PR, 1985. Extinction coefficients of chlorophyll *a* and *b* in N, N-dimethylformamide and 80% acetone [J]. *Plant Physiology*, **77**: 483—485

Johnson GN, 2005. Cyclic electron transport in C3 plants: fact or artifact [J]. *Journal of Experimental Botany*, **56**: 407—416

Johnson GN, 2011. Physiology of PSI cyclic electron transport in higher plants [J]. *Biochimica et Biophysica Acta*, **1807**: 384—389

Klüghammer C, Schreiber U, 1994. An improved method, using saturating light pulses, for the determination of photosystem I quantum yield via $P700^{+}$-absorbance changes at 830 nm [J]. *Planta*, **192**: 261—268

Klüghammer C, Schreiber U, 2008. Saturation pulse method for assessment of energy conversion in PSI [J]. *PAM Application Notes* (*PAN*), **1**: 11—14

Kim SJ, Lee CH, Hope AB *et al.*, 2001. Inhibition of photosystem I and II and enhanced back flow of photosystem I electrons in cucumber leaf discs chilled in the light [J]. *Plant and Cell Physiology*, **42**: 842—848

Kou JC, Takahashi S, Oguchi R *et al.*, 2013. Estimation of the steady-state cyclic electron flux around PSI in spinach leaf discs in white light, CO_2-enriched air and other varied conditions [J]. *Functional Plant Biology*, **40**: 1018—1028

Kramer DM, Johnson G, Kiirats O *et al.*, 2004. New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes [J]. *Photosynthesis Research*, **79**: 209—218

Krieger A, Weis E, 1993. The role of calcium in the pH-dependent control of Photosystem II [J]. *Photosynthesis Research*, **37**: 117—130

Laureau C, De Paepe R, Latouche G *et al.*, 2013. Plastid terminal oxidase (PTOX) has the potential to act as a safety valve for excess excitation energy in the alpine plant species *Ranunculus glacialis* L [J]. *Plant, Cell and Environment*, **36**: 1296—1310

Li XG, Duan W, Meng QW *et al.*, 2004. The function of chloroplastic NAD (P) H dehydrogenase in tobacco during chilling stress under low irradiance [J]. *Plant and Cell Physiology*, **45**: 103—108

Long SP, Bernacchi CJ, 2003. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error [J]. *Journal of Experimental Botany*, **54**: 2393—2401

Miyake C, 2010. Alternative electron flows (water-water cycle and cyclic electron flow around PSI) in photosynthesis: molecular mechanisms and physiological functions [J]. *Plant and Cell Physiology*, **51**: 1951—1963

Munekage Y, Hashimoto M, Miyake C *et al.*, 2004. Cyclic electron flow around photosystem I is essential for photosynthesis [J]. *Nature*, **429**: 579—582

Munekage Y, Hojo M, Meurer J *et al.*, 2002. *PGR5* is involved in cyclic electron flow around photosystem I and is essential for photoprotection in *Arabidopsis* [J]. *Cell*, **110**: 361—371

Murata N, Takahashi S, Nishiyama Y *et al.*, 2007. Photoinhibition of photosystem II under environmental stress [J]. *Biochimica et Biophysica Acta*, **1767**: 414—421

Nandha B, Finazzi G, Joliot P *et al.*, 2007. The role of PGR5 in the redox poising of photosynthetic electron transport [J]. *Biochimica et Biophysica Acta*, **1767**: 1252—1259

Nishiyama Y, Allakhverdiev SI, Murata N, 2011. Protein synthesis is the primary target of reactive oxygen species in the photoinhibition of photosystem II [J]. *Physiologia Plantarum*, **142**: 35—46

Nishiyama Y, Allakhverdiev SI, Yamamoto H *et al.*, 2004. Singlet oxygen inhibits the repair of photosystem II by suppressing the translation elongation of the D1 protein in *Synechocystis* sp. PCC 6803 [J]. *Biochemistry*, **43**: 11321—11330

Nishiyama Y, Yamamoto H, Allakhverdiev SI *et al.*, 2001. Oxidative stress inhibits the repair of photodamage to the photosynthetic machinery [J]. *EMBO Journal*, **20**: 5587—5594

Niyogi KK, Grossman AR, Bjorkman O, 1998. Arabidopsis mutants define a central role for the xanthophyll cycle in regulation of photosynthetic energy conversion [J]. *Plant Cell*, **10**: 1121—1134

Niyogi KK, Shih C, Chow WS *et al.*, 2001. Photoprotection in a zeaxanthin and lutein deficient double mutant of *Arabidopsis* [J]. *Photosynthesis Research*, **67**: 139—145

Oguchi R, Terashima I, Chow WS, 2009. The involvement of dual mechanisms of photoinactivation of photosystem II in *Capsicum annuum* L. plants [J]. *Plant and Cell Physiology*, **50**: 1815—1825

Oguchi R, Douwstra P, Fujita T *et al.*, 2011. Intra-leaf gradients of photoinhibition induced by different color lights: implications for the dual mechanisms of photoinhibition and for the application of conventional chlorophyll fluorometers [J]. *New Phytologist*, **191**: 146—159

Ohnishi N, Allakhverdiev SI, Takahashi S *et al.*, 2005. Two-step mechanism of photodamage to photosystem II: step one occurs at the oxygen-evolving complex and step two occurs at the photochemical reaction center [J]. *Biochemistry*, **44**: 8494—8499

Okegawa Y, Kagawa Y, Kobayashi Y *et al.*, 2008. Characterization of factors affecting the activity of photosystem I cyclic electron transport in chloroplasts [J]. *Plant and Cell Physiology*, **49**: 825—834

Oxborough K, Baker NR, 1997. Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components-calculation of qP and F_v'/F_m' without measuring F_o' [J]. *Photosynthesis Research*, **54**: 135—142

Peltier G, Cournac L, 2002. Chlororespiration [J]. *Annual Review of Plant Biology*, **53**: 523—550

Shikanai T, 2007. Cyclic electron transport around photosystem I: genetic approaches [J]. *Annual Review of Plant Biology*, **58**: 199—217

Shikanai T, Endo T, Hashimoto T *et al.*, 1998. Directed disruption of the tobacco *ndhB* gene impairs cyclic electron flow around photosystem I [J]. *Proceedings of the National Academy of Sciences of the United States of America*, **95**: 9705—9709

Streb P, Josse EM, Gallouët E *et al.*, 2005. Evidence for alternative electron sinks to photosynthetic carbon assimilation in the high mountain plant species *Ranunculus glacialis* [J]. *Plant, Cell and Environment*, **28**: 1123—1135

Takahashi S, Bauwe H, Badger MR, 2007. Impairment of the photorespiratory pathway accelerates photoinhibition of photosystem II by suppression of repair but not acceleration of damage processes in *Arabidopsis* [J]. *Plant Physiology*, **144**: 487—494

Takahashi S, Murata N, 2008. How do environmental stresses accelerate photoinhibition [J]. *Trends in Plant Science*, **13**: 178—182

Takahashi S, Milward SE, Fan DY *et al.*, 2009. How does cyclic electron flow alleviate photoinhibition in *Arabidopsis* [J]. *Plant Physiology*, **149**: 1560—1567

Takahashi S, Badger MR, 2011. Photoprotection in plants: a new light on photosystem II damage [J]. *Trends in Plant Science*, **16**: 53—60

Teicher HB, Møller BL, Scheller HV, 2000. Photoinhibition of photosystem I in field-grown barley (*Hordeum vulgare* L.): induction, recovery and acclimation [J]. *Photosynthesis Research*, **64**: 53—61

von Caemmerer S, Farquhar GD, 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves [J]. *Planta*, **153**: 376—387

Wang P, Duan W, Takabayashi A *et al.*, 2006. Chloroplastic NAD (P) H dehydrogenase in tobacco leaves functions in alleviation of oxidative damage caused by temperature stress [J]. *Plant Physiology*, **141**: 465—474

Yamori W, Evans JR, von Caemmerer S, 2010. Effects of growth and measurement light intensities on temperature dependence of CO_2 assimilation rate in tobacco leaves [J]. *Plant, Cell and Environment*, **33**: 332—343

Yamori W, Sakata N, Suzuki Y *et al.*, 2011. Cyclic electron flow around photosystem I via chloroplast NAD (P) H dehydrogenase (NDH) complex performs a significant physiological role during photosynthesis and plant growth at low temperature in rice [J]. *Plant Journal*, **68**: 966—976